EFFECTS OF COLCHICINE ON THE CLEAVAGE OF THE FROG'S EGG (RANA PIPIENS)

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The most striking biological effect of the alkaloid drug, colchicine, is its capacity, when applied in the proper concentration, to arrest mitosis in the metaphase (Dustin, 1934, 1935). Under certain conditions, especially in plants, this drug has also been shown to be effective in producing polyploidy, doubtlessly due to its ability to inhibit to a greater or lesser degree the orderly sequences of the mitotic process (Blakeslee and Avery, 1937; Nebel and Ruttle, 1938).

There have been a great many studies of the effect of physical and chemical changes in the external environment on the developing egg of the frog. Some of the early results obtained, especially by centrifuging (O. Hertwig, 1897), and by exposure to high temperatures (O. Hertwig, 1898), rather closely parallel the effects produced by treatment with colchicine. In the present report the observations are limited to the changes induced by relatively short exposures to the drug applied almost immediately after fertilization.

Methods

The eggs of *Rana pipiens* were obtained and inseminated by the method of Rugh (1934). After a quarter of an hour the eggs were flooded with water and in another quarter of an hour were separated by dissection but the jelly was not removed. They were then put into the colchicine solutions. In later experiments the solutions were made up and used in the dark, except for a red light, since colchicine is affected in solution by light (Lits, 1936). The experiments were conducted at 8° and 20° C.

Description

In the first series of experiments, the colchicine solutions were made up in concentrations ranging from 1 : 1000 to 1 : 1,000,000 and the eggs were left in the solutions continuously. In the 1 : 1000 solution all cleavage activity was suppressed, and eventually disintegration set in. In the 1 : 10,000 solution the first cleavage took place as in the controls, the second cleavage set in at the normal time but showed some irregularities.

Soon after this, the membranes of most of the eggs seemed to be tightly stretched, appearing as though an abnormal amount of water had been absorbed. The cleavage grooves became more and more shallow. No further cleavage took place, and four and a half hours after fertilization no evidences of cleavage were visible except the presence of light streaks on the animal hemisphere. These streaks, which were due to the absence of superficial pigment, coincided with the position of the cleavage furrows. They appeared to have been caused by the movement of pigment deeper into the egg in association with the development of the clefts.

In the 1 : 100,000 solution of colchicine, the first, second, and third cleavages frequently took place as in the controls. Shortly after the initiation of the third cleavage, many of the eggs showed diminished grooves, the others continued dividing as in the controls. On the following day, some of those in which the grooves were fading showed no trace of cleavage except the light streaks, while others had the animal pole region largely cellular with the vegetative hemisphere uncleaved. The proportion of cellular to non-cellular material varied considerably. These were all dead on the third day.

In the 1 : 1,000,000 solution, the early cleavage was like that in the controls, no fading being evident except possibly on the vegetative halves. The second day embryos varied from completely normal to those with a small cell cap in the region of the animal pole, the rest of the egg being uncleaved. These embryos also, even those that appeared normal, were dead on the third day.

It is evident from these preliminary experiments that the eggs varied considerably in their susceptibility to colchicine, and that with a continuous exposure it was relatively toxic. A 1 : 100,000 exposure for one hour was found to give a fair number of abnormal forms without too high mortality and even a concentration of 1 : 10,000 for the same length of time was also satisfactory, although the mortality was somewhat greater. Most of the eggs in both these concentrations gave rise to normal embryos. The following studies, then, were made on selected individuals showing the characteristic abnormalities.

It is extremely difficult to interpret fully many of the abnormalities obtained by the treatment with colchicine. It was not possible to secure an orderly and progressive series of developmental stages as in normal development. The wide range of susceptibility of the eggs to the drug and the diverse effects obtained make it necessary to consider each egg individually and attempt to relate the conditions in earlier cleavages to the morphological patterns found in the more advanced embryos. In general the segmentation of treated eggs becomes meroblastic, with the

cap of cells produced in the region of the animal pole resting upon a more or less completely undivided yolk.

Early Cleavage

Reference has already been made to the fading of cleavage. Observations on living eggs and sections show that it takes place first at the vegetative pole, and sometimes a furrow extending halfway down the egg is left (Figs. 1, 2, and 3), while in other cases no trace of the cleavage remains, or only a shallow depression may persist at the surface of the animal pole. In some in which no external groove is left a cleft inside the egg can be seen in sections. In rare instances the first cleavage may persist even on the vegetative hemisphere, though subsequent cleavages are restricted in their persistence to the animal hemisphere. The nuclei may go on dividing although there is no cytoplasmic division. In several cases in which the early cleavage furrows had completely disappeared, a curious sort of delayed or secondary cleavage appeared at about the time when the controls were in the blastula stage (three days at 8° C.). In such cases a few relatively small cells were outlined on the surface, and contained one or more large vacuolated nuclei. More similar nuclei appeared in the main body of the egg.

The significant result of the arrest or retardation of the early mitosis and the secondary fading of the early cleavage furrows, especially in the vegetative hemisphere, is the production of an early blastula in which definitive cells tend to be restricted to the animal hemisphere. The degree of restriction of cell cleavage is variable. In extreme cases only a small cap of cells is formed but all gradations from this condition to almost complete cleavage of the yolk of the vegetative half may be encountered.

Blastulae

In many embryos with only a small cellular area in the region of the animal pole a stage comparable to the blastula is not attained. The cells divide progressively but do not assume any regular pattern. They are usually loosely grouped above the undivided yolk mass without the formation of a definite segmentation cavity (Figs. 4 and 6). This condition was observed most frequently in embryos which had developed at the lower temperature, 8° C.

In others, a modified blastula, consisting of a cellular roof which is separated more centrally from the uncleaved yolk by a segmentation cavity or blastocoele, is formed. This cavity apparently may be secondarily enlarged by the progressive vacuolation of the adjoining yolk mass (Fig. 7). Below the cavity are the free nuclei of the yolk, becom-

ing larger, more abnormal, and more irregularly spaced toward the vegetative pole. They are usually surrounded by dense accumulations of pigment.

In embryos in which approximately half of the material is cellular the blastula is frequently more complete with a cellular floor of variable thickness. The roof is often differentiated into two layers comparable to the outer, epidermal and inner, nervous layer. Occasionally, however, part of the animal half may fail to undergo segmentation while a considerable amount of the yolk is cleaved. In such cases the degree of primary segmentation that may have occurred in the yolk is difficult to determine. A study of living animal-hemisphere embryos showed that the first two cleavages could often be seen on the surface of the vegetative pole and subsequently faded. Whether or not these cleavages

EXPLANATION OF PLATE

Figures 1 to 9 are photomicrographs taken at several magnifications. The magnification was varied in order to bring out significant details or to include sufficient area to show relationships. In all instances the solutions of colchicine were applied for one hour, beginning thirty minutes after insemination.

PLATE I

Explanation of Figures

Figs. 1 and 2. Modification of early cleavage; 4 hours, 20 minutes after insemination; temperature 20° C.; colchicine, 1 : 10,000.

Fig. 3. Early cleavage; 5 hours, 20 minutes after insemination; temperature 20° C.; colchicine, 1 : 10,000. The plane of section is oblique to the polar axis giving a false impression of the degree of cleavage of the vegetative hemisphere.

Fig. 4. External view of late cleavage showing restriction of cleavage to the animal half of the egg; 72 hours after insemination; temperature 8° C.; colchicine, 1 : 100,000.

Fig. 5. Partial embryo; 48 hours after insemination; temperature 20° C.; colchicine, 1 : 100,000. From sections it appears that the first cleavage persisted and then cell division was restricted in one blastomere more than in the other.

Fig. 6. Late atypical cleavage; 72 hours after insemination; temperature 8° C.; colchicine, 1 : 100,000. Cleavage is sharply restricted to a cap of cells in the region of the animal pole. Large, numerous yolk-nuclei with concentrations of pigment about them are scattered through the uncleaved yolk.

Fig. 7. Animal-hemisphere blastula; 48 hours after insemination; temperature 20° C.; colchicine, 1 : 100,000. Only the roof of the blastocoele is cellular. The floor is composed of unsegmented yolk which is highly vacuolated. The cellular configuration (on the right) possibly represents the beginning of gastrulation by involution.

Fig. 8. A transverse section of the upper half of an abnormal gastrula; 48 hours after insemination; temperature 20° C.; colchicine, 1 : 100,000. Neural plate, notochord, mesoderm and an incomplete layer of entoderm are present. The underlying yolk is undivided and ventrally an area of degeneration is seen.

Fig. 9. An area from the cellular roof of a blastula; 48 hours after insemination; temperature 20° C.; colchicine 1 : 100,000. The nuclei of this embryo are of two distinct sizes. The smaller are of normal size. The larger may be due to tetraploidy.

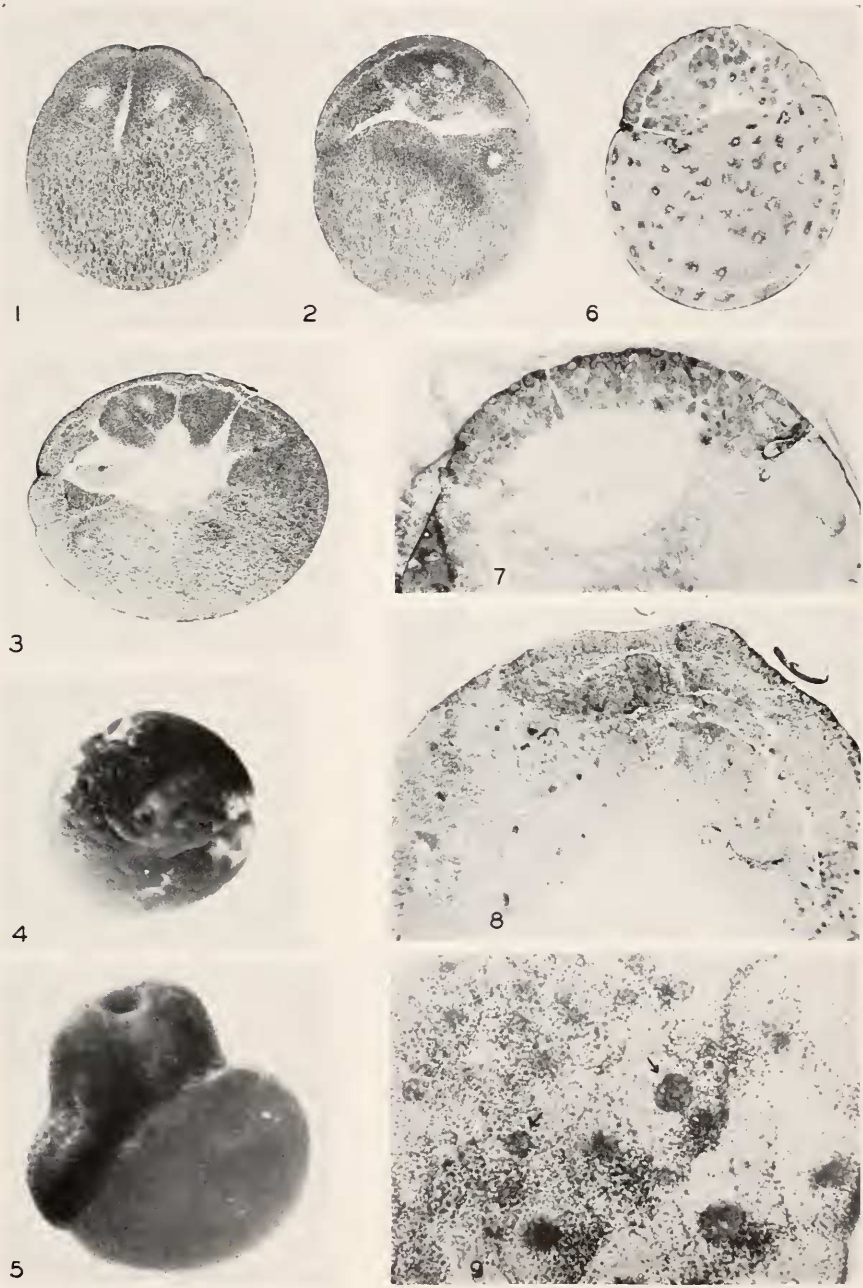

PLATE I

actually passed completely through the yolk was not determined, but it seems fairly safe to conjecture that they had not. The formation of the cellular floor of the blastocoele may have occurred simultaneously with cleavage of the cells nearer the animal pole but there is evidence that in many cases the cleavage of the yolk occurs secondarily, probably through the progressive organization of cells from a region comparable to the germ-ring of normal embryos. As far as it is possible to judge from sections, the process of cellulation occurs around free nuclei directly adjacent to previously formed cells. In these regions the free nuclei are more likely to be normal in size and shape than those at a greater distance from the zone of definitive cells, i.e., nearer the vegetative pole. The cells formed are at first more or less spherical and sometimes extracellular yolk remains between them.

In many instances there was considerable disturbance of the distribution of pigment especially at the margin of the polar cap. Frequently the polar pigment was carried down as irregular streamers over the vegetative hemisphere leaving an irregular border between the animal and vegetative halves. In other instances the local disappearance of pigment on the surface of the animal hemisphere appeared to be due to its being carried deeper into the egg. Degenerative changes may be involved.

Gastrulae

It seems probable in the embryos in which segmentation is restricted to a cap of cells at the animal pole, that gastrulation could not occur. However, even in these, there is a tendency for the marginal cells of the cap to show a regional separation from the uncleaved yolk suggesting the initiation of gastrulation. In more complete blastulae, gastrulation may set in between the cleaved and uncleaved material. More often the yolk is cellular in the immediate region of the invagination. However, the evidence on the process of gastrulation is not conclusive. Externally the blastopore lip is straight and does not seem to be extended laterally very far around the circumference of the egg. In cases in which the yolk is not cleaved at the point of gastrulation the process seems to be limited to simple involution with the cells migrating to form a more or less continuous layer under the roof of the blastula. These cells are usually comparatively small and heavily pigmented, resembling those of the superficial epidermal layer. There does not seem to be any superficial down-growth in the region of gastrulation but laterally and anteriorly there is considerable overgrowth of a single layer of epidermal cells over the undivided yolk.

In embryos with cleaved yolk at the point of gastrulation there is usually a sac-like invagination which extends progressively into the blas-

tocoele and rests on the upper surface of the undivided yolk. In such cases the blastocoele is eventually obliterated and a small archenteron is established.

Later Development

Many embryos die after the initiation of the modified gastrulation, probably because of their inability to carry the process to any degree of completion. However, several survived in which gastrulation appeared to be represented primarily by involution. In these a disc-like blastoderm was developed which consisted of a neural plate with rudimentary neural folds, notochord, tissue occupying the position of dorsal mesoderm and a thin, sometimes incomplete layer of underlying entoderm (Fig. 8). A careful study of serial sections fails to yield any evidence of invagination. Caudally the epidermal cells, forming a free margin, rested directly upon the uncleaved mass of yolk. The yolk-filled tissue lateral to the notochord is interpreted as being derived by the progressive cellulation of the yolk adjacent to its junction with the margin of the blastula roof. The cells of the notochord and entoderm were relatively small and heavily pigmented. This, however, does not constitute an irrefutable argument for their origin by involution as the distribution of pigment is very irregular in these abnormal embryos. Furthermore, even in normal embryos the cells of this region contain considerable pigment, possibly related to their metabolic activity and more rapid multiplication.

Usually the segmentation of the animal hemisphere occurs uniformly and the partial embryos tend to be symmetrical. In some cases, however, the restriction of cleavage is unequal and asymmetrical embryos are produced. Such an embryo is illustrated in Fig. 5. It appears that in this case the first cleavage persisted and then the cell division of the region of the animal pole on one side was restricted more than on the other. Embryos do not survive beyond the neural fold stage.

Nuclear Size

In about half of the animal hemisphere embryos cells were found that were much larger than normal. Those generally occurred in groups and might be either epidermal or deeper-lying cells. They had correspondingly large nuclei (Fig. 9). Mitoses were observed but they were seldom favorable for chromosome counts due to the dense concentration of pigment granules about the spindle figure. Polar views adequately demonstrated that the number of chromosomes in the large cells was greater than normal but the exact ratio could not be established. It would seem quite probable that these represent tetraploid conditions. In

several embryos, one in particular, sectioned at late cleavage, the cells were of varied sizes, and the mitoses were very irregular. Eccentric, distorted monasters often at the sides or corners of the cells were frequent. Such conditions probably explain the variation in nuclear size.

In some cases, especially in eggs which had developed at 8° C., segmentation was restricted to a few superficial cells. In these, the adjacent area was characterized by regularly spaced, small centrospheres with associated asters and surrounded by pigment granules. Many of these were not associated with chromosomes or nuclear material and have been interpreted as cytasters.

Yolk and Free Yolk-nuclei

In the uncleaved portions of the egg the yolk underwent regular changes which were probably degenerative in character. The yolk platelets disappeared locally and large vacuoles were formed as the yolk products went into solution. Progressive coalescence of vacuoles produced large fluid-filled spaces which were distributed irregularly through the non-cellular portion of the egg (Fig. 7).

The free nuclei, especially in the areas more distant from the cleaved portion of the animal pole, became progressively larger and more irregular. In the earlier cleavage stages they appeared to undergo normal mitosis but in the blastula and later stages the mitoses became progressively abnormal and large, irregularly-lobed nuclei appeared. These nuclear complexes were usually surrounded by dense aggregations of pigment granules. Some nuclei are vacuolated and stain very lightly while others are massive and stain densely. These giant nuclei, however, are not characteristic only of colchicine-treated embryos. They were also observed by Hertwig (1897) in the meroblastic segmentation of the frog's egg under the influence of centrifugal force.

Summary

The treatment of the developing frog's egg with colchicine (concentrations from 1 : 10,000 to 1 : 100,000) in amounts sufficient to retard mitosis or cause a temporary arrest of the process, results in a varying proportion of the eggs exhibiting a meroblastic type of cleavage. The degree of restriction of cleavage to the animal-pole region varies from the formation of a small cap of cells to a condition in which cleavage may include almost the entire egg. The level of development and completeness of the embryo are directly correlated with the extent of restriction of cleavage. Such animal-hemisphere embryos may not be capable of attaining even a modified blastula stage. In many blastulae

only the roof of the segmentation cavity is cellular. The process of gastrulation is always greatly disturbed and in extreme cases may be limited to a modified involution. Frequently, neural plate with beginning neural folds, notochord, dorsal mesoderm and entoderm are differentiated. Embryos rarely develop beyond this level.

In many embryos there are scattered areas in which the cells are large and possess correspondingly large nuclei. These are tentatively interpreted as the result of tetraploidy, a phenomenon commonly produced in plants by colchicine. The free yolk-nuclei are characteristically abnormal.

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